IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Harrington, *et al.*

Application No.: 09/513,997

Filed: February 26, 2000

For: **COMPOSITIONS AND METHODS FOR NON-TARGETED ACTIVATION OF ENDOGENOUS GENES**

Group Art Unit: 1643

Examiner: Brunovskis, P.

Attorney Docket No.: 0221-0003Q

Declaration Under 37 C.F.R. § 1.132

Commissioner of Patents
Washington, D.C. 20231

Sir:

The undersigned, John J. Harrington, declares and states:

1. I am an inventor of the above-captioned patent application, U.S. Application No. 09/513,997, filed February 26, 2000, entitled "Compositions and Methods for Non-Targeted Activation of Endogenous Genes." I am the subject of the attached Curriculum Vitae and author of the publications shown on the list attached thereto. On the basis of the information and facts contained in these documents, I submit that I am an expert in the field of non-homologous recombination, eukaryotic gene expression and gene cloning and am qualified to speak on the skill and knowledge of the person of ordinary skill in these fields.

2. I have read and understand the subject matter of the above-captioned application. I have read and understand the Office Action dated February 14, 2000, rejecting claims 81, 83–88, 92, and 101–103 under 35 U.S.C. § 112, first paragraph, on the grounds that it would have required an undue burden of experimentation to practice the claimed invention. It is my opinion, based on the scientific evidence and reasoning below, that the methods that are the subject of the rejected claims could have been made and used by the person of ordinary skill in the art, as of the filing date of September 26, 1997 (the earliest effective filing date), by routine and ordinary experimentation, using the Applicants' specification and general knowledge in the art as a guide.

3. It is my opinion, based on the scientific evidence and reasoning set forth below, that the methods that are the subject of the rejected claims provided a practical, real-world use. I assert this because the methods provide a way to use cells to produce protein in an animal and using cells to produce protein in an animal had a practical, real-world use as of the Applicants' earliest effective filing date.

4. It is my further opinion that the person of ordinary skill in the art reading Applicants' specification would have immediately appreciated that producing protein from Applicants' cells in an animal was useful, as of the Applicants' earliest effective filing date. I assert this because cell-based protein production in an animal was known by the art to be useful and the claimed methods were disclosed as a way to provide cell-based protein production in an animal.

5. As a rationale for the rejection, the Examiner has stated that the specification discloses only one real-world use for the claimed method: cell therapy. I do not agree with this statement because the specification discloses cell-based protein production in an animal and there was real-world use for cell-based protein production in various contexts besides cell therapy. Some of these will be discussed further below in this Declaration. In addition, the specification also discloses the isolation and purification of protein produced in an animal by the cells of the invention. This disclosure clearly demonstrates a utility distinct from cell therapy, since cell therapy does not involve subsequent purification of the protein following expression *in vivo*. Isolation and purification of proteins produced in an animal had real-world use. Thus, the specification does disclose a process with real-world use in addition to use for cell therapy.

The Examiner has also asserted that he is unaware of any well-established utility for the claimed method except cell therapy. I, therefore, point out that there was well-established utility for the claimed method for uses in addition to cell therapy.

I understand a well-established utility to be a real-world use that would have been immediately apparent to the person of ordinary skill in the art reading the Applicants' specification. It is my opinion that both non-therapeutic, cell-based protein production in an animal and isolation of proteins produced from cells placed in an animal would have been recognized immediately as having practical use by the person of ordinary skill in the art.

6. My opinions and conclusions in this Declaration are supported by evidence in the form of scientific references that I will discuss in the paragraphs that follow. These references show that there were non-therapeutic uses for introducing a cell into an animal to produce a desired protein from the cell. The Applicants' disclosure directs the artisan to introduce Applicants' cells into an animal to produce protein from the cell. Having access to the references, the person of ordinary skill in the art would have readily appreciated that the claimed methods also had non-therapeutic utility.

7. The following references, available as of the earliest effective filing date, show some non-therapeutic practical uses for producing protein from cells introduced into an animal.

8. Brodeur et al., Kints et al., and Stewart et al. demonstrate the utility of expressing a protein from a cell introduced into an animal. The utility is not based on cell therapy. Specifically, each of these authors describes a method for introducing hybridomas into mice or rats to produce large quantities of antibodies. The antibodies are produced from endogenous antibody genes in the hybridomas. The purpose of this work was to optimize conditions for producing antibodies so that the antibodies could be purified. It is stated in these references that there are advantages to producing antibodies *in vivo*. The references thus demonstrates a utility for protein production *in vivo* that does not involve cell-based therapy.

The Applicants' specification discloses and claims methods for expressing desired protein from endogenous genes in eukaryotic host cells and subsequently introducing the cells

into an animal to produce protein *in vivo*. Applicants' specification, in fact, also discloses using hybridomas to express endogenous genes using the methods of the invention. See U.S. Application No. 08/941,223, page 30, line 22 and U.S. Application No. 09/276,820, page 53, line 29. The specification also discloses expression of antibodies using Applicants' methods. See U.S. Application No. 08/941,223, page 22, line 25 and U.S. Application No. 09/276,820, page 43, line 16. The specification also discloses isolating and purifying proteins expressed *in vivo*.

Based on these similarities, it is my opinion that it would have been readily apparent to someone skilled in the art that Applicants' cell lines and hybridomas, expressing endogenous antibodies, could be used to produce antibodies *in vivo*. It is, therefore, my opinion that there was practical, well-established utility for using the claimed method.

9. U.S. Patent No. 5,733,761 describes uses for production of proteins *in vivo*. The uses are not based on cell therapy. Desired endogenous proteins are activated by homologous recombination. In column 3, starting at line 17, it is indicated that the cells introduced into the animal are useful for eliciting antibody production or for immunizing humans or animals against pathogenic agents. The antigens can be used to produce antibodies that are then used for therapeutic or diagnostic purposes. This U.S. patent is a continuation of U.S. Application No. 07/985,586. Accordingly, it contains the same specification. WO 94/12650 is a PCT application claiming priority to U.S. Application No. 07/985,586. The relevant text in the U.S. application, discussed above, is found in the PCT application on page 5. The PCT application was published

in June 1994. Accordingly, this information was available to the person of ordinary skill in the art well before the earliest effective filing date of Applicants' claims.

The patent specification discloses non-therapeutic uses in addition to those discussed directly above. In column 14, starting at line 1, the disclosure discusses implanting cells for agricultural use, for example, meat and dairy production. It is my opinion that this disclosure would have been recognized by the person of ordinary skill in the art to include such uses as the delivery of hormones to an animal through the implanted cells. Line 6 then goes on to discuss the implanted cells as also being useful for eliciting antibody production for immunizing humans and animals against pathogenic agents or for producing antibodies useful for therapeutic and diagnostic purposes. This discussion can be found in the corresponding PCT application on pages 27 and 28.

U.S. Patent No. 5,641,670 has a corresponding PCT application, WO 95/31560, published November 23, 1995. In the U.S. patent, column 3, starting at line 27, the specification discusses the use of homologously recombinant cells to immunize animals or produce antibodies in immunized animals. The patent specification also discusses agricultural uses, citing the production of bovine growth hormone for dairy production. See column 4, lines 37–39. Starting at line 47, the specification also discusses *in vivo* immunization and use of the cells to produce antibodies for diagnostic and therapeutic purposes. In column 18, line 40, the disclosure discusses implanting the cells for agricultural uses (i.e., meat and dairy production).

Accordingly, both U.S. Patent Nos. 5,641,670 and 5,733,761, and their corresponding PCT applications, demonstrate utility for production of protein *in vivo* that is not based on cell therapy. It is my opinion that it would have been readily apparent to the person of ordinary skill in the art that the protein-producing cells disclosed in Applicants' specification had the same non-therapeutic utility. It is my opinion, therefore, that the claimed method had practical, well-established utility.

10. Shaw et al. demonstrates the utility of expressing a protein from a cell *in vivo* for other than cell-based therapy. The reference describes the introduction of cells expressing IL-4 and IL-10 into a mouse. The purpose of this work was to produce IL-4 and IL-10 protein *in vivo*. Another purpose of this work was to test the biological activity of the proteins in a disease model. Finally, the authors state that *in vivo* production of protein from introduced cells allows them to produce the proteins at a specific site in the animal, as opposed to systemic delivery of an injected protein.

The Applicants' application also describes introducing cells expressing a protein of interest into an animal to produce protein. The Applicants' specification describes activation and expression of a variety of proteins, including IL-4 and IL-10. See U.S. Application No. 08/941,223, page 22, line 30; page 23, line 10; and page 48, line 16 and U.S. Application No. 09/276,820, page 43, lines 21–23.

Based on these similarities, it is my opinion that using the claimed method to produce IL-4 and IL-10 *in vivo* would have been readily apparent. It would also have been apparent from this reference that producing protein in an animal by the present method is useful to test the biological activity of the protein *in vivo*. It is my opinion, therefore, that the claimed method had practical, well-established utility.

11. Chen et al. demonstrates the utility of expressing a protein from a cell *in vivo* for other than cell therapy. Specifically, the reference describes a method for introducing the nerve growth factor gene into normal fibroblasts, and subsequently introducing the cells expressing NGF into the nucleus basalis magnocellularis (i.e. a region of the brain) of rats. Following implantation, the rats were tested using a Morris water maze to assess their spatial memory ability. The authors show that expression of NGF *in vivo* can reverse naturally occurring age-related memory loss. The authors state that production of protein in an animal "can be used both to explore basic biological questions concerning the structure and function of the brain or as a form of somatic gene therapy. A principal advantage of this approach is the local intraparenchymal delivery of factors to responsive cells, which allow one to examine the effects of the factors on specific populations of cells. Additionally, following the implantation of the transfected cells there is no need for any further invasive procedure, such as the chronic infusion of various factors into the cerebral ventricles by osmotic minipump." Thus, it was appreciated that *in vivo* expression of a protein is useful to define a biological process and potentially as a therapeutic. This art also appreciated the advantages of expressing a protein *in vivo*, as opposed to introducing the purified protein into the animal.

Applicants' specification describes introducing cells expressing a protein of interest into an animal to produce the protein. It also describes activation and expression of a variety of proteins, including nerve growth factors. See U.S. Application No. 08/941,223, page 23, line 6; page 25, line 24; and page 48, line 22 and U.S. Application No. 09/276,820, page 43, line 28. It further describes the use of primary cells and fibroblasts to express a protein of interest. For disclosure of primary cells, see U.S. Application No. 08/941,223, page 30, lines 2 and 13–15 and U.S. Application No. 09/276,820, page 53, lines 9 and 20–22. For fibroblasts, see U.S. Application No. 08/941,223, page 30, lines 7 and 21 and U.S. Application No. 09/276,820, page 54, line 15. It also describes use of any eukaryotic cell, including rat cells. For rat cells, see U.S. Application No. 09/276,820, page 10, line 5 and page 54, line 2.

Based on these similarities, it is my opinion that using the claimed method to produce nerve growth factor *in vivo* would have been readily apparent. Furthermore, it is my opinion that a person of skill in the art would have appreciated the utility of *in vivo* protein expression using the present method to study biological processes including memory and cognition. It is my opinion, therefore, that the claimed invention had practical, well-established utility.

12. Garver et al. demonstrates the utility of expressing a protein from a cell *in vivo* for other than cell therapy. Specifically, the reference describes a method for introducing the human alpha 1-antitrypsin (alpha 1AT) gene into normal mouse fibroblasts, and subsequently

introducing the cells expressing alpha 1AT into the peritoneal cavities of mice. The authors show that human alpha 1AT could be detected in the sera and epithelial surface of the lungs, and that upon recovery, the mouse fibroblasts continued to express alpha 1AT four weeks following introduction into the animal. The authors state that production of protein in an animal is useful as a model for gene therapy and as an "approach to study the *in vivo* effects of such hormones and growth factors."

Applicants' specification describes introducing cells expressing a protein of interest into an animal to produce protein. It also describes activation and expression of a variety of proteins, including alpha 1AT. See U.S. Application No. 08/941,223, page 22, line 24; page 23, line 3; and page 48, line 19 and U.S. Application No. 09/276,820, page 43, line 26. It also describes the use of primary cells and fibroblasts to express a protein of interest. It describes use of any eukaryotic cell, including mouse cells. See U.S. Application No. 09/276,820, page 10, line 5 and page 54, line 2.

Based on these similarities, it is my opinion that a person of skill in the art would have recognized the utility of using the Applicant's method to produce alpha 1AT *in vivo*. Furthermore, it is my opinion that a person of skill in the art would have appreciated the utility of *in vivo* protein expression using the present method to "study the *in vivo* effects of such hormones and growth factors," or other genes. It is my opinion, therefore, that the claimed method had practical, well-established utility.

13. McNiece et al. demonstrates the utility of producing protein from a cell placed in an animal for other than cell therapy. This reference reports the introduction of a cell line, WEHI-3, into mice to produce large amounts of IL-3. IL-3 was expressed from the endogenous IL-3 gene in the cell line. Following introduction into the animal, IL-3 activity was detected in both the sera and ascites fluids of the mice. The IL-3 protein was subsequently purified. In the last paragraph, page 1074, the reference states "The WEHI-3 tumor-bearing mice may thus provide a model for the study of the effects *in vivo* of SF and IL-3 on bone marrow cells." The reference thus demonstrates a utility for protein production *in vivo* other than for cell-based therapy.

Applicants' specification discloses introducing cells expressing a protein of interest into an animal to produce protein. The protein can optionally be purified. It also describes activation and expression of a variety of proteins, including cytokines, and specifically interleukins. For interleukins, see above. For cytokines, see U.S. Application No. 08/941,223, page 22, line 24 and U.S. Application No. 09/276,820, page 43, line 15. It also describes the use of a variety of cell lines similar to WEHI-3.

Based on these similarities, it is my opinion that a person of ordinary skill in the art would have recognized that Applicants' method could be used to produce IL-3 *in vivo*. It is my opinion, therefore, that the claimed method had practical, well-established utility.

14. Ishihara et al. demonstrates the utility of expressing a protein from a cell placed in an animal for other than cell therapy. The reference describes the *in vivo* production of a protease by introducing a tumor cell line, AH109A, into rats. The protease was produced from the endogenous protease gene in the tumor cell line. The purpose of the experiment was to assess the effect of protease expression on tumor cell invasiveness. The protease was also isolated from serum protein and purified 1150-fold to assess and characterize the protease produced *in vivo*.

The Applicants' specification describes introducing cells expressing a protein of interest into an animal to produce protein. The protein can optionally be purified. It also describes activation and expression of a variety of proteins. These include, but are not limited to, proteases, including TPA, urokinase, and protein C. For TPA, see U.S. Application No. 08/941,223, page 22, line 27 and page 48, line 14 and U.S. Application No. 09/276,820, page 43, line 19. For urokinase, see U.S. Application No. 08/941,223, page 23, line 6 and page 48, line 22 and U.S. Application No. 09/276,820, page 43, line 28. For protein C, see U.S. Application No. 08/941,223, page 23, line 4 and page 48, line 20 and U.S. Application No. 09/276,820, page 43, line 27. It also describes the use of a variety of tumor cell lines. These can include a hepatoma cell line, Hep G2, similar to the hepatoma AH109A tumor cell line. For Hep G2, see U.S. Application No. 08/941,223, page 30, line 20 and U.S. Application No. 09/276,820, page 53, line 27. The specification states that cell lines useful for activating endogenous genes can be derived from any tissue. Liver cells and hepatoma cells are specifically cited. For hepatoma cell lines, see U.S. Application No. 08/941,223, page 30, line 21 and U.S. Application No.

09/276,820, page 53, line 28. For liver cells, see U.S. Application No. 08/941,223, page 30, line 4 and U.S. Application No. 09/276,820, page 53, line 11.

Based on these similarities, it is my opinion that a person of skill in the art would have recognized the utility of using the claimed method to produce proteases, such as TPA, urokinase and protein C, *in vivo*. It is my opinion, therefore, that the claimed method had practical, well-established utility.

15. Bronson et al. demonstrates the utility of expressing a protein from a cell *in vivo* for other than cell therapy. The reference reports introducing the bcl-2 gene into mouse embryonic stem cells by homologous recombination. The embryonic stem cells were injected into blastocysts, and subsequently introduced into a pseudopregnant mouse using standard transgenic procedures. Bcl-2 was used as a test gene to establish the feasibility of this transgenic approach.

The Applicants' application describes introducing cells expressing a protein of interest into an animal to produce protein. The application describes activation and expression of a variety of proteins. The present invention describes the use of a variety of cell types including cells isolated from an embryo and stem cells. For embryo cells, see U.S. Application No. 08/941,223, page 30, line 6 and U.S. Application No. 09/276,820, page 53, line 13 and page 54, line 14. For stem cells, see U.S. Application No. 08/941,223, page 30, line 8 and U.S. Application No. 09/276,820, page 53, line 14 and page 54, line 16.

Based on these similarities, it is my opinion that a person of skill in the art would have recognized the utility of using the present method to produce a desired protein, as a test gene for transgenics, *in vivo*. It is my opinion, therefore, that the claimed method had practical, well-established utility.

16. The cited references cumulatively show various art-known uses of cell-based expression of a desired protein in an animal besides cell-based therapy. This evidence demonstrates real-world and well-established utility for cell-based protein expression in an animal that is not cell-based therapy. Since the referenced methods were useful and would have been recognized as useful, Applicants' claimed methods also would have been useful and would have been recognized as such.

Furthermore, because *in vivo* cell-based protein production was not confined (in the literature) to one or two specific proteins or classes of protein, I believe that the person of ordinary skill in the art would have realized that *in vivo* cell-based expression could be useful for any number of proteins. This being the case, they would have also realized that Applicants' methods would also be useful for a desired protein and not just a limited class.

SUMMARY

In summary, the literature shows that cell-based protein production in an animal was useful in a variety of contexts unrelated to cell-based therapy. Therefore, non-therapeutic methods using Applicants' cells to produce protein in an animal also were useful.

The relevant literature on non-therapeutic uses for cells expressing protein in an animal was available to the person of ordinary skill in the art at the earliest effective filing date. Therefore, the person of ordinary skill in the art would have known of these uses as of this date. Accordingly, as of this date, it would have been readily apparent that Applicants' cells could be used non-therapeutically. Thus, there was a well-established non-therapeutic utility for Applicants' methods.

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EDUCATION

1994-1998	Case Western Reserve University, Cleveland, OH Post-doctoral Research in Human Molecular Genetics
1989-1994	Stanford University, Stanford, CA Ph.D. in Cancer Biology
1985-1989:	University of California, San Diego, San Diego, CA B.A. in Biochemistry and Cell Biology (Cum Laude)

RESEARCH EXPERTISE

Protein Biochemistry	Protein purification, gene overexpression, assay design, enzyme kinetics, protein structure-function analysis, identification of novel enzymatic activities
Molecular Biology	Molecular gene cloning, PCR, cDNA and genomic library construction, nucleic acid enzymology, vector construction, Southern and Northern Blot analysis

RESEARCH AND MANAGEMENT EXPERIENCE

1995-present	Executive Vice President, Chief Scientific Officer, and Director, Athersys, Inc. Responsible for directing all research and development activities at the Company, including development of the Company's proprietary technology platforms. Along with the Board of Directors, responsible for oversight on all matters related to Company operations, including budgetary, legal, and strategic issues.
1994-1998	Post-doctoral Research Associate, Case Western Reserve University Constructed Human Artificial Chromosomes. Developed assays for characterization of human artificial chromosomes and the genetic elements required for chromosome function.

1989-1994	Graduate Research Associate, Stanford University. Dissertation Research: Purification, characterization, and molecular cloning of the FEN-1 family of structure-specific endonucleases. Additional Research: Developed cellular and cell-free assays for V(D)J recombination. Thesis Advisor: Dr. Michael Lieber
1988-1989	Research Assistant, Scripps Clinic and Research Foundation. Purification and characterization of Protein C Inhibitor from human plasma. Principal Investigator: Dr. John Griffin

MEETINGS AND ABSTRACTS

List available upon request

PUBLICATIONS

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PATENTS

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U.S. patent # 5,874,283. Mammalian flap-specific endonuclease (1999); **Harrington, J. J.**, Hsieh; C.L., M.R. Lieber.

U.S. patent # 5,695,967. Method for stably cloning large repeating units of DNA (1997); Van Bokkelen; G.B., **Harrington; J. J.**, and H.F. Willard.

REFERENCES

Available upon request.